the DNA in dead cells in these mutants is actually less condensed and more persistent than in the *nuc-1* strain. Since the *ced-1* and *ced-2* strains have normal endodeoxyribonuclease activity (*8*), we conclude that the enzyme does not gain access to the DNA in these cells. In a formal sense, the *ced-1* and *ced-2* products act before the *nuc-1* enzyme. In particular, it suggests that the endonuclease is normally provided by the lysosomes of the engulfing cell and not by the dying cell. It leaves open the intriguing question of what biochemical machinery is used by dying cells to reach the refractile stage characteristic of unengulfed deaths.

Chalfie and Sulston (*9*) have described a dominant mutant [*el611 (mec-4)*] in which a class of six homologous mechanosensory neurons die after initial differentiation. These deaths differ from normal programmed cell deaths in that the cells never become refractile and, very frequently, a large nonrefractile vacuole forms around the dead cell. We examined the degeneration of these neurons in double mutants homozygous for *el611* and either *ced-1* or *ced-2* and found no differences from *el611* mutants alone. This suggests that the wild-type *ced-1* and *ced-2* products are not involved in clearing these abnormal deaths. Similarly, these products do not speed the removal of cells killed unnaturally by the laser microbeam technique (*10*).

Most deaths occur in neuronal lineages and many of the dying cells would be expected to be neurons. Death may occur before (*4*) or after extension of neurites (*1*). In certain cases, we have a good guess for the potential fates of these cells. For example, six sex-specific neurons (four male-specific neurons and two hermaphrodite-specific neurons) are produced in embryos of either sex but only neurons appropriate to the sex of the embryo are retained; the others undergo selective death (*1*). Here we surmise that the cells are fated to be neurons of a particular class with sex-specified death as an overriding fate.

Ellis and co-workers (*11*) recently discovered an additional gene involved in cell death by selecting an apparent revertant of *ced-1*. Mutations in *ced-3* block the initiation of cell deaths and allow doomed cells to differentiate and assume their presumptive underlying fates.

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The Genus-Specific Antigen of *Chlamydia:* Resemblance to the Lipopolysaccharide of Enteric Bacteria

Abstract. *A strong immunological cross-reaction between a major glycolipid antigen of* Chlamydia *and the innermost (Re) core of the lipopolysaccharide of enteric bacteria was demonstrated with the aid of mutants in which the Re structure is exposed. The chlamydial glycolipid resembled the Re lipopolysaccharide in molecular size, solubility, and endotoxic properties and may thus be functionally equivalent to lipopolysaccharide, an essential and characteristic component of the outer membrane of Gram-negative bacteria.*

Chlamydiae are important pathogens of man and many animals, yet their biological nature has been elusive and indeed they have only recently been recognized as bacteria. They have an unusual developmental cycle (*1*) with an intracellular growth form and an extracellular infective form. In both forms the chlamydial cells are surrounded by two membrane layers (*2*), an arrangement typical of Gram-negative bacteria; however, the importance of this morphological feature is difficult to evaluate in organisms lacking the most distinctive layer of the bacterial cell envelope, the peptidoglycan. The presence of lipopolysaccharide (LPS), a typical constituent of the outer membrane of Gram-negative bacteria, has been suggested in chlamydiae on the basis of the pyrogenic properties of chlamydiae and Gram-negative bacteria and because of the immunological relatedness of their lipid components (*3, 4*). The sugar, 3-deoxy-*manno*-octulosonic acid (KDO, or ketodeoxyoctanoic acid), is a constituent of the LPS in most Gram-negative bacteria (*5*). The same sugar has been found in the "genus-specific" glycolipid antigen of chlamydiae, present in both known chlamydial species (*Chlamydia trachomatis* and *C. psittaci*) and throughout the growth cycle (*6*). We report that this glycolipid resembles in several parameters and most notably in its immunological properties the inner core of enterobacterial LPS. These data strongly support the view of the chlamydial outer membrane as equivalent to the outer membrane of usual Gram-negative bacteria.

The structure of LPS has been most thoroughly studied in the enteric bacteria *Salmonella* and *Escherichia coli* (*5*). The LPS molecule consists of three different domains (Fig. 1). The lipid A part is responsible for the endotoxic activity of

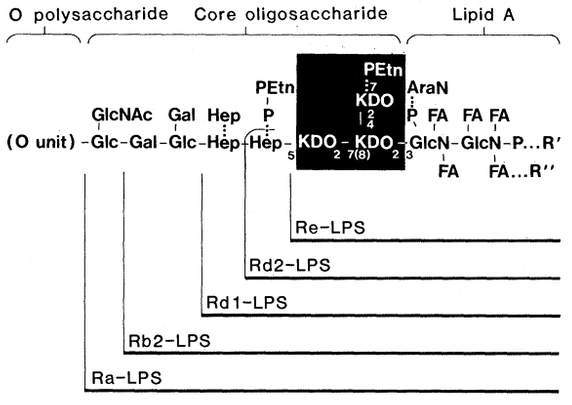

Fig. 1. Schematic structure of *Salmonella* LPS (*5, 16*). The linkages in the KDO region are partly inferred from studies with *E. coli* (*16*). The KDO-trisaccharide is shaded. The LPS of wild-type bacteria has the complete structure, whereas rough mutants have various abbreviated forms (Ra to Re), depending on the biosynthetic block in each case (*8*). Less than stoichiometric substitutions are shown by dotted lines. Abbreviations: *Ac*, acetyl; *Etn*, ethanolamine; *FA*, fatty acid; *KDO*, 3-deoxy-D-*manno*-octulosonic acid; *P*, phosphate; *R'* and *R''*, variable substituents.

LPS; it is conserved in structure, and in the bacteria is embedded in the outer leaflet of the outer membrane. The core oligosaccharide is somewhat more variable but usually consists of a KDO-containing innermost region, a heptose region, and a hexose region. The most distal part, called O polysaccharide, is highly variable in structure and the site of the somatic (O) antigens. The immune response of man or animals injected or infected with these bacteria is mainly directed to the O polysaccharide, which in some way shields the core (*7*). Mutations affecting various steps in the synthesis of LPS (*8*) lead to defective (R) forms of LPS (Fig. 1). In the most defective known R chemotype, called Re, the LPS has only lipid A and the KDO oligosaccharide.

We extracted the Re-LPS from an Re mutant of *Salmonella typhimurium* (*8*) with a mixture of phenol, chloroform, and petroleum ether (PCP), a method developed for R-form LPS (*9*). By applying the same method to *C. trachomatis* type L2, we obtained a glycolipid preparation that we called CT-GL. When Re-LPS and CT-GL were subjected to electrophoresis in sodium dodecyl sulfate and polyacrylamide gel (SDS-PAGE) and stained with silver nitrate, a single spot was observed in both cases, migrating at a similar rate and corresponding to proteins of a molecular mass of approximately 7000 (*10*). Both contained KDO as measured by the thiobarbituric acid method (*11*), the CT-GL containing approximately 5 percent and the Re-LPS, 15 percent.

These preparations were used as antigens in an enzyme immunoassay (EIA) (*12*) to measure antibodies in various antiserums to *Salmonella* and *Chlamydia* (Table 1). High antibody titers to both antigens were seen in the serum of rabbits hyperimmunized with Re mutants of *S. minnesota* (*7*) and *S. typhimurium* (*8*), with Re-LPS from the latter, or with *C. trachomatis* or *C. psittaci*. By contrast, antibodies were not detected to these antigens in normal rabbit serum or serums of rabbits hyperimmunized with a series of R mutants ranging from Ra to Rd2 (Fig. 1) (only the antiserum to Rd2 is shown in Table 1). It is of special interest that even the Rd2 did not provoke antibodies to Re-LPS although the Rd2-LPS consists of the Re structure "capped" by one heptose unit only.

The two antigens (CT-GL and Re-LPS) gave parallel titers for each serum whether the immunizing agent was of chlamydial or of salmonella origin. Because both *Chlamydia* species evoked this cross-reaction the antigen is genus-specific. To further examine the extent of the cross-reaction, we tested other antigens in the EIA: Re-LPS from Re mutants of *E. coli* and *Proteus mirabilis* (*13*), and a glycolipid extracted with ether and acetone (*14*) [the antigen used in the conventional complement fixation (CF) test for *Chlamydia*] from *C. trachomatis*. All of these preparations behaved like CT-GL or Re-LPS with the rabbit serums of Table 1.

To test whether human infection by *Chlamydia* would provoke antibodies with a similar cross-reactivity, we obtained serums from healthy adults and from several patients convalescing from chlamydial infections. All of the serums from patients gave positive results in the CF test for *Chlamydia*, the titers ranging from 16 to 256. The patients all had high or relatively high EIA antibody titers against both the CT-GL and Re-LPS antigens (Table 1). Serums from healthy adults had EIA antibody titers ranging from less than 100 to 200 and CF titers of 8 or less. These preliminary data suggest that Re-LPS might be a useful tool in the serological diagnosis of chlamydial infections.

One of the reactive components of the cross-reaction (the Re-LPS) is a small molecule whose structure is nearly completely known (Fig. 1). It is also known that antibodies to Re-LPS are almost exclusively directed against the oligosaccharide moiety rather than the lipid A part of the molecule (*7*). Aminoarabinose cannot be important in the cross-reaction because one of the reactive Re-LPS (that of *E. coli*) lacks this substituent (*15*). Thus the LPS structures reacting with antiserums to Re (Table 1) must be restricted to the KDO-oligosaccharide-phosphorylethanolamine part of the molecule (shaded area in Fig. 1), and the immunodeterminant shared by *Chlamydia* and *Salmonella* must be either this structure or a part of it. In keeping with this, KDO was present in the CT-GL preparation. We have no information of whether or not the phosphorylethanolamine group linked to the KDO-oligosaccharide would participate in the cross-reaction; however, only a fraction (perhaps 10 percent) of Re-LPS molecules carry this substitution (*16*). To definitively identify the immunodeterminant, inhibition studies with KDO-containing oli-

Table 1. Antibodies to *Chlamydia trachomatis* glycolipid (CT-GL) and *Salmonella typhimurium* Re-LPS measured by enzyme immunoassay (EIA). The antigens were extracted with a mixture of phenol, chloroform, and petroleum ether (*9*) from *C. trachomatis* type L2, strain Bu434 (*20*) or heat-killed bacteria of the *S. typhimurium* Re-type mutant SL1102 (*8*). For the EIA (*12*), the antigens were used at a concentration of 5 μg/ml (CT-GL) or 1 μg/ml (Re-LPS) to coat the wells of 96-well polystyrene plates by overnight incubation at 37°C. Rabbits were immunized with repeated injections of the agents indicated (for the antiserums to *Chlamydia* the first injection was with Freund's complete adjuvant). The patients' disease was identified as of chlamydial origin on the basis of the clinical picture and isolation of *Chlamydia* or positive serological findings. Dilutions of the serums were then applied to the wells, and their binding was detected, after repeated washings, by alkaline phosphatase–conjugated antiserum to rabbit immunoglobulin G (Orion Diagnostica, Helsinki, Finland). The antibody titer was expressed as the reciprocal of the serum dilution giving the absorbance value of 0.3.

Source of immunogen	EIA titer to	
	CT-GL	Re-LPS
Rabbit antiserum		
Salmonella minnesota		
mR595 (*7*), Re, whole bacteria	2000	1500
Salmonella typhimurium		
SL1102 (*8*), Re, whole bacteria	2900	900
SL1181 (*8*), Rd2, whole bacteria	< 100	< 100
SL1102 (*8*), Re, LPS-porin complex (*21*)	1100	2600
Chlamydia trachomatis		
Bu434 (*20*), type L2, Triton X-100 extract	7000	900
UW200, type D (*22*)	600	1000
UW204, type G (*22*)	4400	4500
Chlamydia psittaci		
ATCC VR-656	6800	1600
Pooled rabbit serum, unimmunized	< 100	< 100
Pooled human serum, ten healthy individuals	< 100 to 200	< 100 to 200
Human serum from patients with		
Pneumonia, *C. psittaci*	2200	3200
Pelvic inflammatory disease, *C. trachomatis*	1100	2100
Nongonococcal urethritis, *C. trachomatis*	550	250
Lymphogranuloma venereum, *C. trachomatis*	4600	900

gosaccharides would be necessary. However, the acid lability of the linkages between the KDO molecules makes this difficult. The chemical identity of the chlamydial molecule bearing the cross-reactive antigen is still unknown. In the PCP isolation procedure, the CT-GL behaved like rough LPS but other glycolipids could show similar solubility properties. In SDS-PAGE, it also behaved like Re-LPS (*10*). Schramek *et al.* (*4*) compared a PCP extract of *C. psittaci* with the LPS of a rickettsia (*Coxiella burneti*) and concluded that it contained lipid A; unfortunately, the rickettsial LPS is not well characterized. Endotoxic properties of *Chlamydia* characteristic of lipid A are pyrogenicity in rabbits and the ability to gelate a *Limulus* amoebocyte lysate (*3, 4*). We tested our CT-GL preparation for the latter property and obtained a positive gelation reaction with concentrations of ≥ 0.5 ng/ml (*17*). Together these data suggest that the chlamydial glycolipid is structurally and functionally related to Re-type LPS. We believe that the demonstration of LPS as a component of *Chlamydia* will facilitate studies of the changes believed to take place in the chlamydial cell envelope during its intracellular growth cycle (*18*). It is probable that the biologically active LPS also has a role in the pathogenesis of chlamydial infections.

Note added in proof: The structure of the KDO-region of Re-LPS has recently been reexamined and may contain only a KDO-disaccharide (*19*). This does not alter the conclusions in this report.

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Transcriptional Regulation of Globin Gene Expression in the Human Erythroid Cell Line K562

Abstract. *The effect of hemin on the rate of synthesis and the level of globin messenger RNA's in the human erythroid cell line K562 was examined by means of cloned hybridization probes specific for each of the human embryonic, fetal, and adult globin genes. Hemin increases both the rate of transcription and the level of accumulation of ζ-, ε-, γ-, and α-globin messenger RNA's by a factor of 3 to 5. Thus, hemin induction of globin gene expression in K562 cells is at the level of transcription.*

Erythroid cell lines that are inducible for globin gene expression provide useful models for studying eukaryotic gene regulation (*1–3*). The most thoroughly studied of these lines are mouse erythroleukemia (MEL) cells, which are virally transformed erythroid precursor cells thought to be arrested on or before the proerythroblast stage (*1*). Treatment of MEL cells with dimethyl sulfoxide (DMSO) or various other chemical agents results in terminal erythroid cell differentiation and a 10- to 50-fold increase in the accumulation of adult α- and β-globin proteins (*4*).

An inducible human erythroid cell line, K562, was established from a patient with chronic myelogenous leukemia (*2*). Although K562 was derived from a pregranulocytic cell (*2*), it was subsequently shown to display erythroid cell specific markers, suggesting that the line is a multipotent hematopoietic precursor (*5*). Treatment of these cells with sodium butyrate, hydroxyurea, or hemin resulted in an increase in red cell–specific proteins including hemoglobin (*5–7*). The globin chains synthesized are primarily the embryonic α- and β-like globins, ζ and ε, respectively, and the fetal γ-globin. Low amounts of adult α-globin are also produced, but no adult β-globin is observed (*6, 7*).

In the case of MEL cells the accumulation of globin messenger RNA (mRNA) and hemoglobin after induction by DMSO results at least in part from transcriptional activation of adult globin genes (*8*). In order to investigate the level at which globin gene expression is controlled in K562 cells, we have examined the effect of hemin treatment on the level of globin mRNA's and on the rate of globin gene transcription.

We estimated the levels of specific globin mRNA's by RNA blotting (*9*), and the rates of globin gene transcription by an in vitro nuclear transcription assay in combination with a "dot blot" procedure (*8, 10, 11*). In the latter assay, labeled nascent RNA is prepared from isolated nuclei and hybridized with recombinant plasmids carrying specific DNA sequences bound to nitrocellulose filters. Such a nuclear transcription assay supports elongation of initiated RNA polymerase II transcripts, but does not permit reinitiation (*10*). Thus, the amount of radioactivity incorporated into transcripts that hybridize to a specific DNA fragment reflects the number of RNA polymerase molecules engaged in the transcription of the corresponding fragment of nuclear DNA.

The hydridization probes used are indicated on the map of the human α- and β-globin gene clusters (Fig. 1). The β-globin gene cluster contains an embryon-